

# Profile distribution and seasonal dynamics of water-extractable carbohydrate in soils under mixed broad-leaved Korean pine forest on Changbai Mountain

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**Abstract:** Carbohydrate represents an important part of the soil labile organic carbon pool. Water soluble carbohydrate drives the C cycle in forest soil by affecting microbial activity and hot water extractable carbohydrate is thought related to soil carbon sequestration due to the association with soil aggregation. In a temperate forest region of northeast China, Changbai Mountain, we investigated the abundance, spacial distribution, and seasonal dynamics of cool and hot-water extractable carbohydrate in soils under mixed broad-leaved Korean pine forest. The concentrations of cool-water extractable carbohydrate (CWECH) in three soil layers (0–5, 5–10, 10–20 cm) ranged from 4.1 to 193.3 g·kg<sup>-1</sup> dry soil, decreasing rapidly with soil depth. On an annual average, the CWECH concentrations in soils at depths of 5–10 and 10–20 cm were 54.2% and 24.0%, respectively, of that in the 0–5 cm soil layer. CWECH showed distinct seasonal dynamics with the highest concentrations in early spring, lowest in summer, and increasing concentrations in autumn. Hot-water extractable carbohydrate (HWECH) concentrations in three soil layers ranged from 121.4 to 2026.2 g·kg<sup>-1</sup> dry soil, which were about one order of magnitude higher than CWECH. The abundance of HWECH was even more profile-dependent than CWECH, and decreased more rapidly with soil depth. On an annual average, the HWECH concentration in soils 10–20 cm deep was about one order of magnitude lower than that in the top 0–5 cm soil. The seasonality of HWECH roughly tracked that of CWECH but with seasonal fluctuations of smaller amplitude. The carbohydrate concentrations in cool/hot water extracts of soil were posi-

tively correlated with UV<sub>254</sub> and UV<sub>280</sub> of the same solution, which has implications for predicting the leaching loss of water soluble organic carbon.

**Keywords:** forest soil; cool-water extractable carbohydrate; hot-water extractable carbohydrate; profile distribution; seasonal dynamics

## Introduction

Carbohydrate, representing 5%–20% of soil total organic matter, is an important part of the soil labile organic carbon pool (Ball et al. 1996). As one of the most degradable soil components, carbohydrate can serve as a sensitive indicator of change in soil total organic carbon (TOC) (Biederbeck et al. 1994; Zhang et al. 2007). The content and properties of carbohydrate influence the transformation and relocation of soil organic and inorganic materials, and are closely associated with the environmental quality of soil (Sleutel et al. 2006). Carbohydrate is also an important aggregation compound in most soils. Yousefi et al. (2008) reported that the stability of soil aggregates was more significantly correlated with hot-water extractable carbohydrate than with soil TOC and changes in carbohydrate can indicate changes in soil aggregation. Because soil aggregation is an important mechanism for stabilization of soil organic matter (SOM), especially in organic rich soils (Six et al. 2000; Lützow et al. 2006), we believe that carbohydrates play a role in soil carbon sequestration.

Soil carbohydrate is mainly derived from plant debris and root secretion, and secondarily from microbial lysis and metabolites. Microbes utilize plant-origin OM to produce microbial carbohydrate and leave it accumulated in the soil (Cheshire 1979). As an indispensable constituent of microbial cells and their main energy source, carbohydrate is closely bound up with soil microorganisms (Piao et al. 2000), and microbial activity is important in consuming and producing carbohydrates in soil (Hishi et al. 2004). Dissolved organic carbon (DOC) in soil might be the most important C source for microbes (Smolander and Kitunen 2002), since most microbial metabolism depends on

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water transport of resources (Metting 1993). The turnover of dissolved organic matter (DOM) driven by microbial activity is considered to be important in cycling of soil C and N (Kalbitz et al. 2000). A large proportion of DOM is composed of carbohydrates (Ghani et al. 2003; Hishi et al. 2004; Bu et al. 2011), and carbohydrate represents the main bio-substance in DOM which is usually readily assimilated and used as an energy source by soil microbes (Dakora and Philips 2002). Current knowledge leads us to the recognition that it is necessary to study the carbohydrates in DOM in the interest of soil C and N cycling, especially to obtain deeper insights into the dynamics of carbon in soils.

The production and consumption of carbohydrates in DOM are thought to be controlled by several soil and environmental factors (Kalbitz et al. 2000). The fresh leaf litter mass, organic layer, plant root exudates (Yano et al. 2000), and microbial lysis (Kaiser et al. 2001) all act as sources of carbohydrate. While microbial consumption (Kaiser et al. 2001), leaching due to soil solution, and adsorption to clay minerals (Cheshire 1985; Boudot 1992; Miltner 1997) all reduce the amount of carbohydrate in DOM. Temperature, water content, dissolved organic nitrogen (DON), and phenols in DOM itself are physicochemical environmental factors that affect the concentration of carbohydrate in DOM indirectly via microbial consumption and lysis (Hishi et al. 2004). Many of the factors controlling carbohydrate in DOM vary both spatially and temporally. Few studies have focused on the seasonal dynamics and profile distribution of carbohydrate in DOM, and the subject is poorly understood.

Soil DOM can be obtained using column leaching (Wagai and Sollins 2002), lysimeter (Qualls 2000; Michalzik et al. 2001), and water extraction methods (Hishi et al. 2004; Zhang et al. 2009; Bu et al. 2011). Different methods yield different DOMs in terms of both quantity and quality. The quantity and the biological nature of soil soluble organic matter are also largely affected by extraction temperature (Sparling et al. 1998; Gregorich et al. 2003). We selected two relevant indices of soluble carbohydrate in DOM or in water soluble OM, viz. cool- and hot-water extractable carbohydrate (abbreviated as CWECH and HWECH, extracted at 25 °C and 100 °C, respectively), which are also used as indices of the *active* soil carbohydrate. Our objectives for this study were to (1) quantify the cool- and hot-water extractable carbohydrate in soils under mixed broad-leaved Korean pine forest in the Changbai Mountains, (2) examine the profile distribution and seasonal dynamics of CWECH and HWECH, and (3) supplement current knowledge of the dynamics of carbon in soils.

## Materials and methods

### Site description

Sampling sites were located on the north slope of Changbai Mountain National Nature Reserve (41°42'45"–42°45'18"N, 127°33'30"–128°16'48"E) at elevations of 730–760 m. The sites were characterized by a montane temperate monsoon climate with precipitation averaging 800 mm·a<sup>-1</sup> and mean annual tem-

perature of 5.6 °C. Snow cover generally occurs from late October to late April. The representational zonal vegetation in this region is characterized by mixed broad-leaved Korean pine forest, which is dominated by Korean pine (*Pinus koraiensis*), with native auxiliary broadleaf tree species including *Acer mono*, *Quercus mongolica*, *Tilia amurensis*, *Fraxinus mandshurica* and others. Large areas of the virgin forest have been well protected and kept intact since the 1960s. Soil type is homogeneous and classified as Baijiang Soil (Typic Albi-Boric Argosols, CST) developed on the Basalt Platform of Changbai Mountain. The soils here are commonly well to imperfectly drained, with an organic horizon (O horizon) about 5 cm thick, a humus horizon (A horizon) about 10 cm and an albic horizon (E horizon) about 20 cm thick.

### Soil Sampling

Five sampling plots (30 × 30 m) of virgin mixed broad-leaved Korean pine forest were established on the north slope of Changbai Mountain National Nature Reserve in autumn 2011, with three randomly selected but afterwards permanent sampling points in each plot. In spring (early May), summer (mid July) and autumn (late September) of 2012, bulk soil core samples were collected from three depths of the mineral soil (0–5, 5–10, 10–20 cm) at each sampling point. In total, 5 plots × 3 points × 3 seasons × 3 depths = 135 mineral soil samples were collected during the study. For each filed core sample, visible materials such as roots and litter were removed manually, and the soil sample was thoroughly transferred and sealed in a plastic bag, and then placed in a portable icebox during transportation to the laboratory. During the field procedure of soil sample collection, the thickness of the O horizon was measured, and the live fine roots of plants were collected in separate plastic bags for later determination in the laboratory of fine root density.

### Chemical analysis

Soil samples were transported to laboratory within 12 h. Each fresh sample was passed through a 2 mm mesh after weighing on the whole to estimate soil bulk density. Samples were then divided into two parts: one part was stored at 0–4 °C until analysis for water extractable carbohydrate and other relevant indices, and the second part was air-dried for TOC and other basic property determinations (Table 1).

Cool-water extraction was achieved by a modified procedure described by Jiang and Xu (2006). Briefly, a 10 g subsample of field-moist soil was shaken (250 r·min<sup>-1</sup>) in 100 ml of distilled water at 25 °C for 30 min, and then centrifuged for 10 minutes at 6000 r·min<sup>-1</sup>. The suspensions were then filtered through a 0.45 µm nitrocellulose membrane. The filtrate was stored at -20 °C before chemical analysis.

Hot water extract was obtained by boiling a 10 g aliquot of field-moist sample in 100 ml distilled water for 60 min (Landgraf et al. 2006). After cooling to room temperature, the filtrate was prepared and stored as described above for cool water extraction.

**Table 1** Soil general properties of the sampling sites

Soil depth cm	Bulk density g·cm <sup>-3</sup>	pH	TOC g·kg <sup>-1</sup>	TN g·kg <sup>-1</sup>	C/N
0–5	0.66 c	5.51 ab	134.11 a	7.26 a	18.47 a
5–10	0.89 b	5.67 a	45.85 b	3.40 b	14.26 b
10–20	1.21 a	5.42 b	8.38 c	0.85 c	9.86 c

**Notes:** Data in the table represent averages of  $5 \times 3 \times 3 = 45$  samples across three seasons for pH, and averages of  $5 \times 3 = 15$  samples collected in autumn for TOC, TN and bulk density. Within a column, different lowercase letters mean significant differences by Duncan's means comparisons,  $p < 0.05$ . Bulk density was estimated with dry soil weight of the core sample, soil pH was determined using an in situ pH meter, soil TOC and TN were determined by dry combustion on an Elementar Vario EL C/N Analyzer (Heraeus Elementar Vario EL, Hanau, Germany).

Total carbohydrate concentrations in filtrate of cool/hot-water extracts were determined using the anthrone-sulfuric acid method described by Grandy et al. (2000), briefly, the filtered solution was diluted properly with water before addition of 10 ml of the anthrone-sulfuric acid reagent, and absorbance of the resulting solution was read at 625 nm using a Spectronic 7221 spectrophotometer (Shanghai Instrument Company). Simultaneously, another aliquot of the filtrate sample was diluted 2–10 times or so, to determine the UV absorbance at 254 nm ( $UV_{254}$ ) and 280 nm ( $UV_{280}$ ), using a UV-vis spectrophotometer (UV-9200, Beijing Rayleigh Instrument Company) with 1 cm quartz cell.

#### Statistical analysis

All the data were treated with Excel 2003, and statistical analyses were conducted using SPSS 18.0 (Statistical Product and Service Solutions).

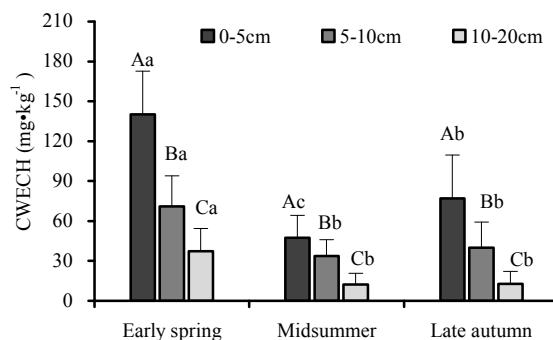
## Results

### Cool-water extractable carbohydrate

Cool-water extractable carbohydrate (CWECH) concentrations ranged from 4.1 to 193.3 mg·kg<sup>-1</sup> dry soil, with considerable differences between soil layers, decreasing rapidly with soil depth (Fig. 1). On an annual average of all five sample plots, the CWECH concentrations in soils from 5–10 cm and 10–20 cm depths were only 54.2% and 24.0%, respectively, of those in the top 0–5 cm soil layer. The spacial variation in horizontal directions (i.e. variation among 15 sampling points at 5 plots) of soil CWECH concentration was also conspicuous, with coefficients of variation (CV%) ranging from 23.2%–74.0%. Variability in the 10–20 cm soil layer was much greater than in the two upper layers (Table 2).

CWECH showed distinct seasonal dynamics. Highest concentrations were found in early spring, followed by sharp declines in summer to the trough when concentrations were about 1/2–1/3 of those in spring, and then increasing again to varying degrees in autumn. Different soil layers exhibited similar sea-

sonality in CWECH concentration, but there were no significant differences between summer and autumn for the two deep layers at 5–10 cm and 10–20 cm (Fig. 1). Spacial variation of CWECH concentrations also varied seasonally. Differences among soil layers were smaller in summer than in spring and autumn, this was true especially for upper soil layers (Fig. 1). The horizontal variation in CWECH concentration for each soil layer was generally smaller in spring than in summer and autumn (Table 2).



**Fig. 1** Vertical distribution and seasonality of cool-water extractable carbohydrate concentrations in the 0–5, 5–10, and 10–20 cm mineral soil layers. For a given season, significant differences (Duncan's means comparisons,  $p < 0.05$ ) between the means of different soil layers are marked with different capital letters. For a given soil layer, significant differences (Duncan's means comparisons,  $p < 0.05$ ) between the means of different seasons are marked with different lowercase letters.

**Table 2** The inter-plot coefficient of variability (CV%) of CWECH concentrations for different soil layers and their seasonal changes (n=15)

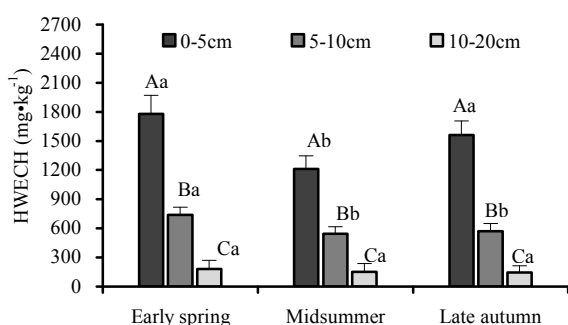
Soil layer	Early spring	Midsummer	Late autumn	Annual average
0–5cm	23.2	35.4	42.3	33.6
5–10cm	32.2	36.7	48.0	39.0
10–20cm	45.5	68.0	74.0	62.5
Profile average	33.6	46.7	54.8	45.0

### Hot-water extractable carbohydrate

Hot-water extractable carbohydrate (HWECH) concentrations ranged from 121.4–2026.2 g kg<sup>-1</sup> dry soil, which were about one order of magnitude higher than CWECH (Fig. 2). The concentrations of HWECH declined with soil depth just as for CWECH but the declines were proportionally greater for HWECH: concentrations in soils at 10–20 cm depths were about one order of magnitude lower than in the top 0–5 cm of soil (Fig. 2 and Fig. 3). In contrast, the horizontal variation of soil HWECH concentration was relatively weak, especially in the upper 0–5 cm and 5–10 cm soil layers where the coefficient of variation ranged from 9.3%–14.2%, only one third of that for CWECH of the same layer (Table 3 and Table 2).

The trend in seasonal variation of HWECH approximated that of CWECH but with much smaller seasonal fluctuations (Fig. 1

and Fig. 2). Early spring was generally the period when HWECH was most abundant in any soil layer, followed by summer when HWECH concentrations declining to varying minor degrees (32%, 26%, 16% average decrement for 0–5, 5–10, 10–20 cm soil layers, respectively), and the autumn rise was only evident in the top 0–5 cm layer. It could also be considered that the seasonality of concentrations of HWECH in deeper soil layers were not so significant, especially in the 10–20 cm layer where soil TOC was particularly low (Table 1). Spatial variations in HWECH, both vertical (between layers) and horizontal (between sapling points), were less pronounced seasonally than for CWECH (Fig. 2, Table 3).



**Fig. 2** Vertical distribution and seasonality of hot-water extractable carbohydrate concentrations in the 0–5, 5–10, and 10–20 cm mineral soil layers. For a given season, significant differences (Duncan's means comparisons,  $p < 0.05$ ) between the means of different soil layers are marked with different capital letters. For a given soil layer, significant differences (Duncan's means comparisons,  $p < 0.05$ ) between the means of different seasons are marked with different lowercase letters.

**Table 3** The inter-plot coefficient of variability (CV%) of HWECH concentrations for different soil layers and their seasonal changes (n=15)

Soil layer	Early spring	Midsummer	Late autumn	Annual average
0–5 cm	10.9	11.3	9.3	10.5
5–10 cm	10.4	13.3	14.2	12.6
10–20 cm	48.2	55.4	47.3	50.3
Profile average	23.2	26.7	23.6	24.5

Correlation between water extractable carbohydrate concentration and UV absorbance

We determined the UV absorbance at 254 nm ( $UV_{254}$ ) and 280 nm ( $UV_{280}$ ) for cool- and hot-water extracts and calculated Pearson's correlation coefficient between UV absorbance and carbohydrate concentration. The carbohydrate concentrations in cool- and hot-water extracts of soil were positively correlated with  $UV_{254}$  and  $UV_{280}$  of the same solution, at significant or highly significant levels, even when analyzed without regard to the influence of soil depth (Table 4).

**Table 4** Pearson correlation coefficients (r-value) between water extractable carbohydrate concentration and UV absorbance of the same extracted solution as calculated for different soil layers over three sampling seasons (n=45)

Soil layer	CWECH (20 °C)		HWECH (100 °C)	
	$UV_{254}$	$UV_{280}$	$UV_{254}$	$UV_{280}$
0–5cm	0.429**	0.322*	0.765***	0.537***
5–10cm	0.343*	0.346*	0.621***	0.589***
10–20cm	0.354*	0.430**	0.524***	0.410**

Significant levels: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (two-tailed).

## Discussion

The content of water-extractable carbohydrate

CWECH concentration in forest soil has rarely been reported, despite the abundant literature dealing with soil DOC or water extractable organic carbon (WEOC). Hishi et al. (2004) measured CWECH values between 12.4 and 88.2  $mg \cdot kg^{-1}$  (averaged  $28.9 \pm 15 \text{ mg} \cdot kg^{-1}$ , expressed as carbohydrate C equivalent) in the top 0–5 cm of soil along a topographic sequence in a cool temperate deciduous forest in Japan. Their CWECH values were much lower than our results for the comparable 0–5 cm soil layer.

The quantity and quality of soluble organic matter in soil are substantially affected by extraction temperature. The hot-water extracts might be more chemically heterogeneous because hot water kills living cells, making microbial biomass components extractable, and also extracts substantial amounts of non-microbial organic matter (Sparling et al. 1998). Gregorich et al. (2003) found that the amount of hot-water (at 80 °C) extractable organic carbon (HWECH) exceeded that for cold-water extractable carbon by a factor of two, which was comparable to our result that HWECH was about one order of magnitude higher than CWECH (Fig. 1, Fig. 2). In this study, the concentrations of HWECH in upper soil layers were higher than or similar to the range previously reported for subtropical forest soils in China (Zhang et al. 2009; Bu et al. 2011). Zhang et al. (2009) measured HWECH concentrations between 166.8–661.5  $mg \text{ C} \cdot kg^{-1}$  in the upper 0–10 cm of soils in four subtropical forests, with an extracting procedure of 24 h at 80 °C. Bu et al. (2011) quantified HWECH concentrations between 102.6–203.6  $mg \text{ C} \cdot L^{-1}$  in hot water extracts (equivalent to 205.2–407.2  $mg \text{ C} \cdot kg^{-1}$  soil) from 0–10 cm soils under four different vegetation types along an elevation gradient in the Wuyi Mountains, by an extracting procedure of only 20 min at 80 °C. The inherent differences between forest ecosystems, soil depths, and extraction procedures might be important factors causing variable results for different researchers.

Variations of water-extractable carbohydrate among three soil depths

Dissolved organic carbon (DOC) or water extractable organic carbon (WEOC) in forest soils are generally profile dependent,

decreasing markedly with soil depth (Boyer and Groffman 1996; Corre et al. 1999; Wang et al. 2002; Wu and Xu 2005; Peichl et al. 2007; Sanderman et al. 2008; Bu et al. 2010; Rosenqvist et al. 2010), especially from surface to subsurface layers, corresponding with the vertical distribution of soil total organic carbon (TOC) (Boyer and Groffman 1996; Corre et al. 1999; Wang et al. 2002). For the Baijiang Soils on Changbai Mountain, organic matter was most abundant in the 0–5 cm layer (Table 1), as were fine roots of plants. The 0–5 cm plus 5–10 cm layers were just equivalent to the humus horizon (A horizon), while the 10–20 cm layer was mostly representative of the E horizon where soil TOC (Table 1) and fine plant roots both decreased to a minimum. This might be the main reason that CWECH and HWECH decrease markedly from 0–5 cm to 5–10 cm, and drastically from 5–10 cm to 10–20 cm (Fig. 1, Fig. 2).

#### Seasonality of water-extractable carbohydrate in soils

The marked seasonal variation in carbohydrates can be explained by several processes: leaching from fresh leaf litterfall, production by plant roots, physicochemical dissolution from solid C affecting the carbohydrate concentration, microbial consumption and exudation, and carbohydrate leaching from mineral soil (Kalbitz et al. 2000). The effects of recent biological processes on carbohydrate production, such as fresh leaf litterfall, microbial exudation and plant root activity, are probably important. Fresh litterfall can generate large amounts of carbohydrates (Qualls and Haines 1992) and a litter bag experiment showed that the remaining weight of soluble carbohydrates in leaf litter of temperate forest declined quickly during the first 5 months (Osono and Takeda 2001). In the Changbai Mountains, late September is the season when fresh leaf litterfall occurs. Early May might be the period when carbohydrates are released from litterfall and accumulate temporarily in soil because this is the post-thaw time when soil microbial activity has not yet recovered and litterfall has only been subject to limited decomposition outside the frozen period during November–April. Therefore, the high concentration of carbohydrates in spring and autumn might be partly due to fresh litterfall.

#### Correlation between water-extractable carbohydrate concentration and UV absorbance

Ultraviolet absorption parameters have been used recently by researchers to characterize the concentration or structural properties of organic matter in soil solution. Absorbance at 254 nm was positively and significantly correlated with the total concentration of organic matter in soil solution (Ge et al. 2003; Luo et al. 2007), and absorbance at 280 nm correlated with the aromaticity of organic matter in the same liquid sample (Zsolnay et al. 1996; Marschner and Kalbitz 2003; Gao et al. 2010). The positive and significant correlation between carbohydrate concentration and UV<sub>254</sub> absorbance (Table 4) means that water extractable carbohydrate, as the main labile component in water extractable organic matter (WEOM), its abundance is essentially associated with or even controlled by the magnitude of the total WEOM

pool. In other words, the magnitude of the total WEOM pool could be reflected by the abundance of water extractable carbohydrate, whether extracted with cool or hot water. Water extractable aromatic or humic substances represent the dominant stable components of WEOM. The positive and significant correlation between carbohydrate concentration and UV<sub>280</sub> absorbance (Table 4) implies a quantitative coherence between the two main components (labile and stable) of WEOM. This relationship holds despite the fact that the percentages of carbohydrate or aromatic substances in WEOM might vary during the year, and that their percentages would inevitably be different in cool- versus hot-water extracts. Water extractable or dissolved aromatic substances are bio-stable and have been considered as the main constituents of soluble organic carbon (SOC) leaching loss (Zsolnay et al. 1996). So we deduced that early spring and mid-summer might be two seasons when much greater SOC leaching losses occur because soluble aromatic substances were reflected abundant in early spring (corresponding high CWECH) when accompanied by snow melting, whereas rainfall is most intense in middle summer despite the relatively low concentration of leachate.

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